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## Review

# Structure, membrane orientation, mechanism, and function of pexiganan – A highly potent antimicrobial peptide designed from magainin

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## ABSTRACT

The growing problem of bacterial resistance to conventional antibiotic compounds and the need for new antibiotics have stimulated interest in the development of antimicrobial peptides (AMPs) as human therapeutics. Development of topically applied agents, such as pexiganan (also known as MSI-78, an analog of the naturally occurring magainin2, extracted from the skin of the African frog *Xenopus laevis*) has been the focus of pharmaceutical development largely because of the relative safety of topical therapy and the uncertainty surrounding the long-term toxicology of any new class of drug administered systemically. The main hurdle that has hindered the development of antimicrobial peptides is that many of the naturally occurring peptides (such as magainin), although active in vitro, are effective in animal models of infection only at very high doses, often close to the toxic doses of the peptide, reflecting an unacceptable margin of safety. Though MSI-78 did not pass the FDA approval, it is still the best-studied AMP to date for therapeutic purposes. Biophysical studies have shown that this peptide is unstructured in solution, forms an antiparallel dimer of amphipathic helices upon binding to the membrane, and disrupts membrane via toroidal-type pore formation. This article covers functional, biophysical, biochemical and structural studies on pexiganan.

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## 1. Introduction

There is a pressing need for the development of novel antimicrobial therapies due to the emergence of antibiotic resistant bacterial strains. In the United States, the Center for Disease Control (CDC) has reported that approximately 1.7 million cases of healthcare-associated infection resulting in 99,000 deaths are occurring annually [1]. Among these the major sites of infection are urinary tract, surgical site, lung and blood stream [1]. The long standing and continuing problem

posed by antimicrobial resistant bacterial strains is exemplified by *Staphylococcus aureus* and *Streptococcus pneumoniae*.

Methicillin resistant *S. aureus* (MRSA) was reported as early as 1961 with wide spread occurrences by 1991 [2,3]. Later, reports of reduced susceptibility to vancomycin were made in 1997 [4] followed by isolation of vancomycin resistant *S. aureus* in 2003 [5]. *S. pneumoniae* serotype 19A was reported in 2007 which is resistant to all US Food and Drug Agency approved antimicrobial agents [6].

The emergence of bacterial strains resistant to most or all of the clinically useful antibiotics has provided the impetus to develop new classes of antibiotics that may combat bacterial resistance more effectively. Antimicrobial peptides (AMPs) show promise as therapeutic agents against a broad spectrum of microbes including bacteria,

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fungi and viruses [7–9]. Widely distributed in multicellular organisms, they form part of the initial line of defense in the innate immune system and are also implicated in the activation of the adaptive immune response against microbes [10]. The innate and adaptive immune effects of mammalian AMPs, such as defensins and cathelicidin-derived peptides, include antimicrobial activity [8,11–13], antiviral activity [14], degranulation of mast cells [15,16], promotion or enhancement of antigen, cytokine and chemokine response [17,18]. Anticancer activities of AMPs have also been reported [19–21].

Although highly diverse in sequence and structure, almost all AMPs share the property of being highly amphipathic, with one face of the peptide being hydrophobic and the other face presenting a cluster of positively charged residues [22,23]. AMPs are often classified based on the structural characteristics of the peptides. These classifications include  $\alpha$ -helical, linear, or disulfide bonded [8]. The number of disulfide bonds ranges from 1 to 4 and results in  $\beta$ -hairpin-like structures [13].

Naturally occurring AMPs with  $\alpha$ -helical structures include cecropins from insects and mammals [24], magainins from frogs [25,26], and cathelicidins from mammals [11,27]. Linear AMPs include indolicidin, a tryptophan rich peptide from cows and PR-39, a proline/arginine rich peptide from pigs [28,29]. The disulfide bond containing AMPs includes polyphemusin [30,31] and tachyplesin [32,33] from horseshoe crab, protegrin-1 from pig [34,35], human- $\beta$ -defensin-3 [13], and  $\alpha$ -defensin (HNP3) from humans [36].

The length of AMPs ranges from ~12 to 50 amino acids making them reasonably easy to synthesize. This has led to a vast number of studies that systematically investigate the importance of amino acid composition, peptide length, net charge, and hydrophobicity on the antibacterial activity of AMPs [37,38]. These investigations and mechanistic studies on gene-encoded AMPs have provided much insight into the mechanisms of AMPs.

Whereas some AMPs have been determined to act intracellularly [39], most appear to function primarily by disrupting bacterial cell membranes [40,41]. Bacterial cell membranes contain predominantly negatively charged phospholipids that give rise to an electrostatic attraction to the highly cationic AMPs. On the other hand, eukaryotic membranes, which contain predominantly neutral phospholipids, are usually less susceptible to disruption by AMPs. In addition the presence of cholesterol in eukaryotic membranes increases the resistance against membrane disruption by AMPs. Upon association with the membrane, unstructured peptides become structured and begin thinning the bacterial membrane and proceed to disrupt the membrane through one of three broadly defined methods. The barrel stave method involves peptide insertion into the membrane parallel to the lipid bilayer normal, the toroidal pore method induces bending of the lipid bilayer resulting in pores in the membrane where lipids tilt in such a way that the lipid head groups define the surface of the pore, and the micellization model results in the degradation of membranes through the formation of lipid encompassed peptides [11,42,43]. There are other mechanisms, generally categorized as carpet mechanism, that destabilize the membrane structure to cause cell death.

There is extensive literature and general reviews on AMPs and their mechanisms. In this article, we have specifically focus on Pexiganan or MSI-78 and its very promising attributes. Within this review we describe the process by which the AMP Pexiganan was developed, the antimicrobial activity of the peptide and the high-resolution biophysical characterization of the structure and mechanism of Pexiganan. The potential therapeutic applications of Pexiganan and further developments of Pexiganan analogs are also discussed.

## 2. Pexiganan or MSI-78

Among the hundreds of gene-encoded and designed AMPs, magainin-2 and its analogs have been very well studied; amino acid sequences are given in Fig. 1. Magainin-2 was co-discovered in 1987 after scientists found that *Xenopus laevis* were able to remain

**Table 1**

Minimum inhibitory concentrations for aerobic and anaerobic bacterial strains and percentage of strains susceptible to inhibition by Pexiganan or ofloxacin from literature values.

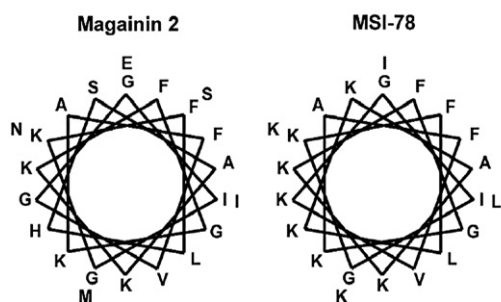
Bacteria strain	MIC Pexiganan ( $\mu\text{g/mL}$ )		% Strains susceptible at $\leq 64 \mu\text{g/mL}$		% Strains susceptible to ofloxacin at $\leq 2 \mu\text{g/mL}$
	Ref. [53]	Ref. [54]	Ref. [53]	Ref. [54]	Ref. [53]
<b>Aerobic bacteria</b>					
<i>Acinetobacter</i> sp.	8	8	100	100	100
<i>Alcaligenes faecalis</i>	N/A	256	100	71	80
<i>Citrobacter diversus</i>	16	8	100	99	100
<i>Citrobacter freundii</i>	16	8	96	100	92
<i>Corynebacterium jeikeium</i>	4	8	100	100	100
<i>Enterobacter aerogenes</i>	16	32	100	100	100
<i>Enterobacter cloacae</i>	32	64	96	92	100
<i>Escherichia coli</i>	16	32	100	100	83
<i>Klebsiella oxytoca</i>	16	16	100	99	100
<i>Klebsiella pneumoniae</i>	16	16	100	100	100
<i>Psuedomonas aeruginosa</i>	16	16	100	99	100
<i>Staphylococcus aureus</i> (MRSA)	64		100		20
<i>Staphylococcus aureus</i> (MSSA)	16	16	100	100	93
<i>Staphylococcus epidermidis</i>	8	8	100	100	100
<i>Staphylococcus haemolyticus</i>	8	8	100	100	100
<i>Streptococcus agalactiae</i>	32	16	100	100	92
<i>Streptococcus pyogenes</i>	32	8	97	99	96
<b>Anaerobic bacteria</b>					
<i>Bacteroides fragilis</i>	6	4	100	100	67
<i>Bacteroides ovatus</i>	6	8	100	100	0
<i>Clostridium perfringens</i>	18	64	100	90	100
<i>Clostridium ramosum</i>	16	16	100	100	0
<i>Clostridium sporogenes</i>	6	16	100	100	100
<i>Peptostreptococcus anaerobius</i>	45	32	100	91	100
<i>Peptostreptococcus magnus</i>	1	8	100	97	0
<i>Prevotella bivia</i>	69	32	78	94	100
<i>Prevotella melaninogenica</i>	51	64	100	91	33

infection-free upon making incisions in the frogs' skin and placing the frog in water containing high levels of microbes [43,44]. Two peptides were isolated from *X. laevis*, magainin-1 and 2. The 23 amino acid magainin-2 was soon found to have broad-spectrum antibacterial and antifungal activity [45,46]. Many synthetic analogs of magainin-2 have been developed to maximize the broad spectrum activity of the peptide in hopes of developing a clinically useful antimicrobial therapeutic agent.

Zasloff et al. discovered that removal of amino acid residues from the N-terminus of magainin-2 resulted in a loss of activity [45]. Omission of residues past Lys4 was particularly detrimental to the activity of the peptide lower the MIC at least 30 $\times$  for *E. coli* in comparison to magainin-2 [45]. Removal of amino acid residues from the C-terminus of the peptide also negatively affected the activity of the peptide significantly [45]. These results suggested that the minimal peptide length was important potentially due to the mechanism of the peptide.

The helical content of the peptide was explored as a potential target for improving the activity of magainin by Chen et al. [46]. Gly to Ala substitution, which were made to increase the stability of the  $\alpha$ -helical structure, resulted in improved activity and attempts to disrupt

**Magainin 1:** GIGKFLHSAGKFGKAFVGEIMKS  
**Magainin 2:** GIGKFLHSAGKFGKAFVGEIMNS  
**MSI-78:** GIGKFLKKAKKFGKAFVKILKK-NH<sub>2</sub>



**Fig. 1.** Amino acid sequences of naturally-occurring magainins (magainin-1 and 2) and MSI-78 or pexiganan, designed based on magainin 2. Helical wheel diagrams of magainin 2 and MSI-78 illustrate the amphipathicity of the peptide in helical form.

the helicity of the peptide by substituting D-amino acids proved to decrease the activity of the peptide [46]. These studies established the importance of the secondary structure of the peptide upon association with the cell membrane [46,47].

Systematic single amino acid mutations to the peptide were performed by Cuervo et al. C-terminal amidation was found to increase peptide activity as well as removal of Glu19 [48]. Poly-lysine and poly-arginine sequences appended to the termini of magainin-2 were also developed with the intent of improving the electrostatic attraction of AMP for anionic bacterial membranes [49]. The resulting peptides did not show increased activity.

With the information available from previous research Zasloff and coworkers of Magainin Pharmaceuticals did an extensive SAR study that resulted in the development of MSI-78 or Pexiganan which entered clinical trials for topical treatment of diabetic foot ulcers [50]. In 1999 the FDA denied approval of pexiganan after completion of two phase III clinical trials that revealed pexiganan was no more effective than already approved treatments for diabetic foot ulcers and required additional clinical trials for consideration [51]. Magainin Pharmaceuticals became Genaera Corporation followed by the recent acquisition of worldwide rights to pexiganan by MacroChem. Improvements in clinical trial design, greater understanding of diabetic foot ulcers and topical anti-infective treatments, and advances in peptide manufacturing keep hopes alive regarding the potential FDA approval of pexiganan [52].

### 3. Activities of Pexiganan

Extensive in vitro studies have been conducted to determine the minimum inhibitory concentration of pexiganan for a diverse array of

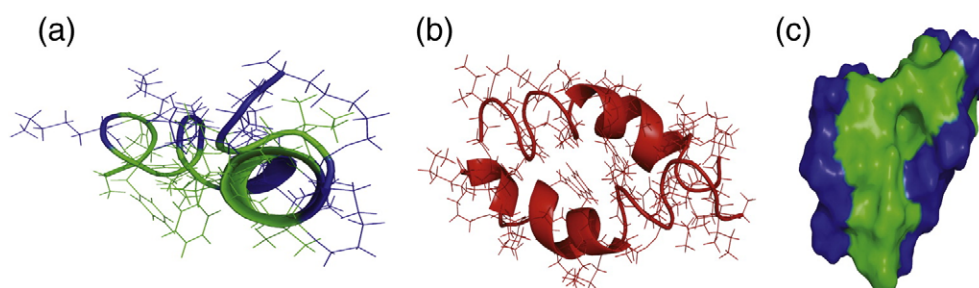
microbes. The major categories of microbes that are of interest as potential targets for Pexiganan have included both aerobic and anaerobic, as well as Gram positive and Gram negative bacteria. Table 1 summarizes the MIC values obtained for Pexiganan from two sources with comparative figures for ofloxacin, the FDA approved drug used as a comparative treatment in the phase III clinical trials of Pexiganan [53,54]. The results clearly show the effectiveness and broad spectrum activity of Pexiganan in vitro. In addition, attempts to generate resistance in bacteria by repeated treatment at subinhibitory concentration of the peptide were unsuccessful. Importantly, *S. aureus*, a bacteria that has quickly developed multiple resistance to current antimicrobial compounds including methicillin and vancomycin [2–5] was included in these attempts and showed no resistance to Pexiganan [54].

Kinetics of Pexiganan cytotoxicity were studied for *E. coli* and *S. aureus* at 50 µg/mL. Colony forming units were reduced to zero by 30 min for *E. coli* and 60 min for *S. aureus* [55]. These results show the rapid onset of activity displayed by Pexiganan in vitro. The broad-spectrum activity, low propensity for generating resistance and quick onset of activity are ideal characteristics for potential therapeutics. The potential toxicity of Pexiganan has been established by measuring the hemolytic activity of the peptide against human red blood cells. Reported numbers suggest that a concentration of at least 250 µg/mL are necessary to induce 100% hemolysis [55–57], much below the MIC for many of the bacterial strains listed in Table 1. This exemplifies the high selectivity against erythrocytes and, in turn, low likelihood of toxicity. In addition, no adverse side effects were reported during the 2 phase III clinical trials described in detail in Ref. [58]. The promising attributes of Pexiganan have led to detailed studies regarding the structure and mechanism of the peptide.

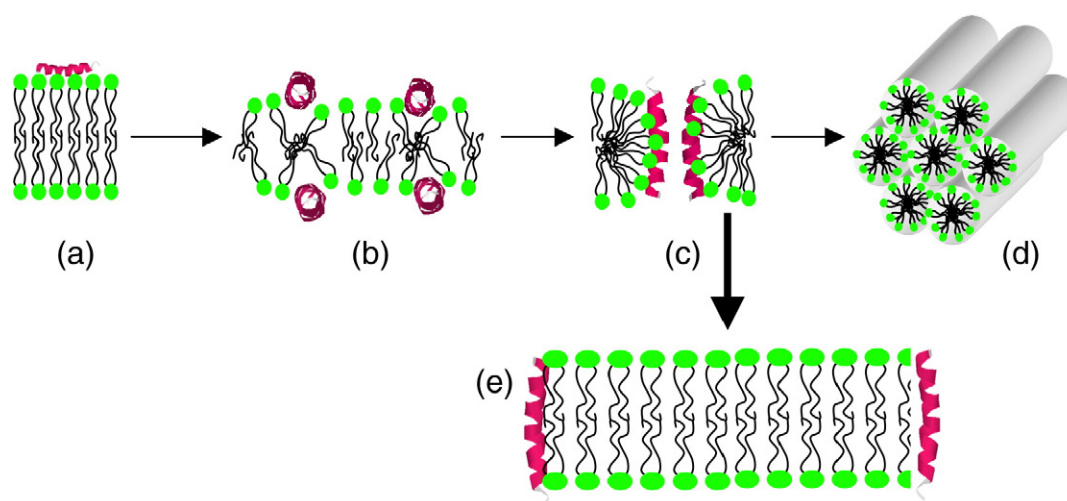
### 4. Structural studies

Solving the secondary structure of an AMP has been considered to be an important step in understanding its function and will be useful in developing potent peptides for pharmaceutical applications. While global structure analysis of AMPs using low-resolution techniques like CD and FTIR usually provides a quick information on the experimental conditions under which a conformational change occur, atomistic-level resolution three-dimensional structures can provide high-resolution information on peptide–peptide and peptide–membrane interactions. Such high-resolution structural information are powerful in understanding the role of individual amino acids in the formation of oligomers in solution or in a membrane environment and in providing insights into the mechanism of cell lysis. Below we cover the structural studies on pexiganan in solution and in model membranes.

Circular dichroism studies have shown that MSI-78 is unstructured in solution and forms an  $\alpha$ -helix in the presence of lipids or detergents [59]; a number of biophysical studies reported similar behavior for magainin-2 and its membrane interaction is also well established



**Fig. 2.** Monomeric (a) and antiparallel dimeric (b) helical structures of MSI-78 determined by NMR experiments in a membrane environment [67]. (c) A surface representation showing the hydrophobic interface (green) and hydrophilic exterior (blue) of the dimeric helical structure. The formation of a dimer is a key step in its activity. Since the dimer has more hydrophilic surface exposed for the membrane interaction and hydrophobic residues are not exposed outside, the selectivity of the peptide towards negatively charged (both Gram positive and Gram negative) bacterial membranes is increased. Therefore, the toxicity of the peptide is further reduced.



**Fig. 3.** Mechanism of membrane disruption by MSI-78. (a) NMR studies have shown that MSI-78 is unstructured in solution and forms a helix in a membrane environment [59,67]. The amphipathic peptide is aligned near the surface of the membrane [59]. (b) Positive curvature strain induced by the peptide is determined from  $^{31}\text{P}$  solid-state NMR on POPE bilayers and differential scanning calorimetry experiments on DiPoPE bilayers [81]. (c) Formation of toroidal pores was determined from solid-state NMR studies [81]. (d) Solid-state NMR studies revealed the formation of normal hexagonal phase structure of lipids at higher concentrations (> 10 mol%) of MSI-78 [59,81]. (e) Solid-state NMR experiments revealed that a couple of weeks old samples exhibited the formation of bicelles and then micellization due to the detergent-like behavior of the peptide [results unpublished].

[60–62]. Shanmugam et al. also showed that Pexiganan is able to adopt a  $\beta$ -turn structure when in methanol or dimethylsulfoxide [63]. The highly cationic nature of the peptide results in a random coil structure in aqueous solution due to electrostatic repulsion between lysine side chains. While this property is similar to most linear AMPs that are not structured in water or ionic solutions, the only human member of the cathelicidin-derived peptides, LL-37, forms a helical structure either in the presence of ions or at high peptide concentration as it forms helical oligomers [64–66]. Upon association with the membrane surface, particularly anionic lipid head groups, a charge balance is achieved and Pexiganan assumes an  $\alpha$ -helical structure [59].

The high-resolution structure, oligomerization state, and orientation of membrane associated Pexiganan has been studied by NMR spectroscopy [59,67]. A dimeric antiparallel  $\alpha$ -helical coiled-coil structure is formed on association with dodecylphosphocholine micelles and bilayers [67]. The interface of the dimer is a 'phenylalanine zipper' composed of three phenylalanine side chains per helix (Fig. 2). The leucine and isoleucine residues near the termini of the helices also pack together at the dimer interface. The importance of the three phenylalanine residues in the self-association of Pexiganan is confirmed by the fact that MSI-594, which lacks 2 of the 3 phenylalanine residues, does not oligomerize in a membrane environment [67]. Solid-state NMR studies on mechanically aligned model membranes and multilamellar vesicles of phospholipids [68], and chemical crosslinking with glutaraldehyde [69], also suggest that Pexiganan self-associates to form dimers in the presence of lipid vesicles. Interestingly, magainin-2 is also a random coil in solution and forms a dimeric antiparallel helical structure in dilauroylphosphatidylcholine vesicles at a higher concentration than that of MSI-78 (Fig. 2) [70]. A recent solid-state NMR study utilized REDOR (rotational echo double resonance) [71] MAS (magic angle spinning) experiments on selectively labeled peptides to determine the backbone conformation of pexiganan in phospholipids bilayers [67]. Structural analysis revealed that the structure of pexiganan is the same in detergent micelles and in lipid bilayers.

## 5. Topology of pexiganan in membranes

In addition to the high-resolution structure, folding and topology (or membrane orientation) of an AMP is essential to fully understand the functional properties of an AMP. For example, the exact membrane

orientation of an AMP can provide insights into the mechanism by which an AMP lyse bacterial cells. Solid-state NMR experiments on aligned samples have been used to obtain this information on several AMPs. Difficulties related to the preparation of fully-hydrated and stable bilayers for this purpose have been overcome by a newly developed naphthalene procedure [72]. This successful preparation of mechanically aligned glass-plate bilayer samples has been vital in the investigation of a number of AMPs [59,64,72,73]. 2D PISEMA (polarization inversion spin exchange at the magic angle) [74] solid-state NMR experiments on mechanically aligned bilayers containing  $^{15}\text{N}$ -labeled pexiganan peptides were used to measure  $^{15}\text{N}$  chemical shifts and  $^1\text{H}$ – $^{15}\text{N}$  dipolar couplings associated with amide sites of the peptide. These NMR parameters revealed that the helical pexiganan is oriented near the surface of the membrane [59]. These results ruled out the barrel-stave type mechanism of membrane disruption (or channel formation) by pexiganan. Similar observations have also reported for magainin-2 peptide [75,76]. On the other hand, based on  $^{19}\text{F}$  [77] and  $^2\text{H}$  [78] solid-state NMR experiments on PGLa embedded in lipid bilayers the authors have suggested that the helical peptide initially binds to the membrane surface and then forms dimers that are tiled by inserting into the hydrophobic region of the membrane. These studies have shown that solid-state NMR experiments on fluid and physiologically-relevant lipid bilayers provide insights into the function of antimicrobial peptides that are difficult to obtain by other means. It should also be mentioned here that care must be taken in interpreting solid-state NMR data obtained from samples that are not physiologically relevant.

## 6. Mechanistic studies

The mechanism of membrane interaction and disruption has been established via fluorescence assays, calorimetric techniques, microscopy, solid-state NMR spectroscopy and neutron diffraction. The consensus is that Pexiganan exerts its antibacterial effect by forming toroidal pores in the bacterial membrane (Fig. 3).

Cell membrane disruption by Pexiganan was confirmed by monitoring the uptake or leakage of fluorescent molecules from either *E. coli* or lipid vesicles, respectively [59]. Pexiganan effectively induced the uptake of ANS into *E. coli* cell membranes as well as inducing the leakage of carboxyfluorescein from POPC/POPG (3:1) vesicles, a model system for bacterial cell membranes. In both studies,



the membranes were maximally affected within 5 min of exposure to Pexiganan, indicating the rapid onset of membrane disruption by the peptide [59].

Isothermal titration calorimetry reveals that the association of Pexiganan to lipid vesicles is exothermic with a binding enthalpy of  $-14.4$  kcal/mol [79] and is in agreement with measurements on magainin-2 which has a  $\Delta H = -17.0$  kcal/mol [80]. The binding enthalpies of cationic peptides have been attributed primarily to the electrostatic interactions between the peptide and the lipid head groups.

The effect of the peptide on lipid bilayers has been very informative of the mechanistic route of Pexiganan [81]. Differential scanning calorimetry (DSC) and NMR spectroscopic studies have pinpointed the formation of toroidal pore formation by Pexiganan in lipid bilayers [81]. DSC experiments in DiPoPE show a concentration dependant increase in the fluid lamellar to inverted hexagonal phase transition of the bilayer which supports the induction of positive curvature strain on the lipid bilayer upon incorporation of Pexiganan [81].  $^{31}\text{P}$  NMR also showed that Pexiganan inhibited the fluid lamellar to inverted hexagonal phase transition and  $^{15}\text{N}$  NMR supporting the formation of toroidal pores in the lipid bilayer due to the orientation of the peptide perpendicular to the bilayer normal [59,81]. Solid-state NMR results from mechanically aligned bilayers were used to determine the toroidal pore geometry in the presence of pexiganan. Solid-state NMR studies on bicelles containing MSI-78 showed the peptide-induced disorders in the hydrophobic region of the lipid bilayer and also the detergent behavior of the peptide [82]. The significant reduction in the  $^{14}\text{N}$  quadrupole coupling observed from the choline head group of the lipid in bilayers containing pexiganan [66] and molecular dynamic simulations [83] revealed the electrostatic interactions between the positively charged residues of the peptide and the phosphate  $\text{O}^-$  atom of the lipid head group.

Membrane thinning effects of Pexiganan have been visually observed using atomic force microscopy (AFM). Mecke et al. present images of supported lipid bilayers composed of DMPC in the presence and absence of Pexiganan [84]. There is an obvious thinning of the bilayer as a function of time in the presence of the peptide.

## 7. Can Pexiganan be made more potent?

Although promising as broad-spectrum antibiotics, MSI-78 and other AMPs are susceptible to proteolysis in vivo by endogenous or bacterial proteases, which may considerably diminish their effectiveness for intravenous applications. Studies on Leishmania pinpoint leishmanolysin as the preventative factor in AMP induced apoptosis of the bacteria [85]. Attempts to overcome this problem by increasing the dose of the AMP often lead to toxic side effects, most notably lysis of red blood cells, which has been attributed to non-specific hydrophobic interactions between the peptide and the eukaryotic cell membrane [86]. Improvements to the stability and/or activity of Pexiganan would be significant for the potential application of Pexiganan, or derivatives thereof, as antimicrobial therapeutic agents.

Derivatives of Pexiganan-related peptides designed to further optimize the stability and activity of the peptide for potential applications in vivo include: acylated analogs [50,87,88] and non-natural amino acid analogs [35,89]. Incorporation of the 12 carbon lauryl group or amino lauryl group to the N terminus of MSI-78 resulted in increased hemolytic activity and no substantial gains in antibacterial activity [87]. Non-natural amino acid analogs, specifically fluorinated amino acids, including hexafluoroleucine and pentafluorophenylalanine [69] have been substituted into MSI-78 for the hydrophobic residues leucine, isoleucine and phenylalanine [35,89]. These substitutions allow for retained helical structure in lipids, micelles, or trifluoroethanol [35,89]. In addition, antimicrobial activity is generally retained or improved in hexafluoroleucine variants with no additional hemolytic activity. Also, the proteolytic stability of the

fluorous peptides is greatly enhanced giving rise to potentially improved bioavailability of the peptides [35,89].  $\beta$ -peptides [90], peptoids composed of poly-N-substituted glycines [91], and cationic oligoureia polymers [92] have also been designed to mimic the structure and amphipathic nature of Pexiganan, but have shown to be not susceptible to proteolytic degradation.

Synergistic capability of Pexiganan has been evaluated against bacteria present in the bloodstreams of neutopenic febrile patients including *P. aeruginosa*, *E. coli*, *S. aureus* (methicillin resistant and methicillin susceptible) and *S. epidermidis* (methicillin resistant and methicillin susceptible) [93]. MSI-78 appears to act in synergy with  $\beta$ -lactams which has been attributed to increased ability of the  $\beta$ -lactams to penetrate the bacterial membrane due to thinning of the membrane in the presence of the AMP [93]. Sepsis rat models have also been used to determine the effectiveness of Pexiganan alone and in combination with  $\beta$ -lactams [94]. The synergistic activity of the Pexiganan/ $\beta$ -lactam combination is observed for the treatment of endotoxic shock. The LPS binding ability of Pexiganan is implicated as a very important aspect of the synergistic effect of these antibiotics due to the association of endotoxin release with  $\beta$ -lactam activity [94].

## 8. Potential applications

The antimicrobial activity of Pexiganan against a broad spectrum of bacterial species makes it a promising candidate for the treatment of bacterial infections. In addition to topical application for the treatment of diabetic foot ulcers as was studied in clinical trials, the above described studies imply the potential applicability of Pexiganan for the treatment of sepsis, particularly in combination with approved antibacterial agents such as  $\beta$ -lactams. The development of non-natural amino acid containing or non-peptidic mimics of Pexiganan could be a key to improving biological stability and bioavailability while retaining the broad-spectrum activity and low toxicity characteristics of Pexiganan.

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